



Human Genome Epidemiology (HuGE) Review

Genetic Variants of Glutathione S-Transferase as Possible Risk Factors for Hepatocellular Carcinoma: A HuGE Systematic Review and Meta-Analysis

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The authors performed a systematic review and meta-analysis to determine the effect of polymorphisms in genes encoding glutathione S-transferases (GSTs), phase II isoenzymes involved in cellular detoxification, on risk of hepatocellular carcinoma (HCC). Fifteen eligible studies were identified: 14 evaluated *GSTM1*; 13, *GSTT1*; three, *GSTP1*; and one each evaluated *GSTM2*, *GSTM3*, *GSTA1*, *GSTA4*, *GSTO1*, and *GSTO2*, respectively. All were case-control studies performed in populations with high (Asian, African) and medium (European) HCC incidence rates. Random-effects meta-analyses suggested a small excess risk of HCC with *GSTT1* null (odds ratio (OR) = 1.19, 95% confidence interval (CI): 0.99, 1.44) and possibly *GSTM1* null (OR = 1.16, 95% CI: 0.89, 1.53) genotypes. Cumulative meta-analyses demonstrated that both pooled estimators generally trended toward a small excess risk with publication of more recent studies. Results for *GSTP1* A313G suggested no excess risk (OR = 0.75, 95% CI: 0.50, 1.15). A number of potentially interesting gene-gene and gene-environment interactions were reported, but these were too few and inconsistent to allow meta-analysis. The overall results suggest that there may be a small excess risk of HCC in individuals with *GSTT1* null and possibly also with *GSTM1* null genotypes. However, given the relatively limited total number of subjects examined and observed between-study heterogeneity, chance could not be excluded.

carcinoma, hepatocellular; epidemiology; genetics; glutathione S-transferase pi; glutathione transferase; humans; liver neoplasms; meta-analysis

Abbreviations: CI, confidence interval; GST, glutathione S-transferase; HCC, hepatocellular carcinoma; OR, odds ratio.

Editor's note: This paper is also available on the website of the Human Genome Epidemiology Network (<http://www.cdc.gov/genomics/hugenet/>).

Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality (1). Hepatocellular carcinoma (HCC) accounts for between 85 percent and 90 percent of all primary liver cancers. There are marked geographic variations in the distribution of HCC, with most cases occurring in either sub-

Saharan Africa or in eastern Asia. Racial and ethnic variations in incidence have been observed even within the same geographic region. Further, in virtually all geographic regions and racial/ethnic groups, men are at a two- to fourfold greater risk of developing HCC (2). Environmental risk factors such as viral hepatitis seem to explain most but not all of these variations.

A very small minority of HCC cases are associated with familial disorders of mendelian inheritance, such as hereditary hemochromatosis or alpha-1-antitrypsin deficiency, whereas the great majority of adult-onset HCC cases are

sporadic, with many having at least one established non-genetic risk factor, such as habitual alcohol abuse, chronic infection with hepatitis B or C virus, or exposure to aflatoxin B₁, a powerful dietary hepatocarcinogen. However, most individuals with these known environmental risk factors never develop cirrhosis or HCC, while a sizable minority of HCC cases develop among individuals without known risk factors.

Genetic variation has been postulated to influence the variable risk for HCC observed both within and across populations. It has recently become possible to perform large-scale epidemiologic studies to evaluate genetic risk factors given rapid advances within the field of genomics, including the completion of the Human Genome Project and a dramatic reduction in the cost of genetic testing.

Currently, the most extensively studied inherited genetic risk factors for HCC are variants of glutathione *S*-transferases (GSTs) (Enzyme Commission (EC) number 2.5.1.18). GSTs are a broadly expressed family of phase II isoenzymes that protect against endogenous oxidative stress, as well as exogenous potential toxins. They detoxify a variety of electrophilic compounds, including oxidized lipid and DNA products generated by reactive oxygen species damage to intracellular molecules (3). Several GST variants are principally expressed in the liver, an organ whose primary functions include detoxification and metabolism. In the liver, inflammation is related to a variety of insults, including hepatitis B virus, alcohol, and hepatitis C virus, which are sources of reactive oxygen species. The liver is also exposed to other carcinogenic by-products as the result of both normal and drug metabolism, as well as exposure to exogenous environmental toxins such as aflatoxin (3–5).

Cytosolic and membrane-bound forms of GST are encoded by two distinct supergene families. At present, eight distinct classes of the soluble cytoplasmic mammalian GST have been identified: alpha, kappa, mu, omega, pi, sigma, theta, and zeta (6). *GSTM1* is one of the genes encoding the mu class of enzymes located on chromosome 1p13.3 and contains 10 exons (7). The theta class of GST enzymes is encoded by the *GSTT1* gene, which is mapped to chromosome 22q11.23 and contains six exons (8). The *GSTP1* gene encodes the pi class of enzymes, the gene is located on chromosome 11q13, and it has nine exons (7).

The *GST* genes are highly polymorphic and frequently indelible. Among the numerous *GST* genes, *GSTM1*, *GSTT1*, and *GSTP1* genes have been extensively examined in association with risk of cancer (6, 9) and clinical outcomes of cancer patients (7, 10–13). The most common variant of the *GSTM1* and *GSTT1* genes is homozygous deletion (null genotype), which has been associated with the loss of enzyme activity and increased vulnerability to cytogenetic damage (14, 15). According to a report from pooled data of 12,525 Caucasians, 2,136 Asians, and 996 African Americans (16), the frequency of the *GSTM1* null genotype is 53 percent (range: 42–60 percent) in Caucasians, 27 percent (range: 16–36 percent) in African-American subjects, and 53 percent (range: 42–54 percent) in Asians. The frequency of the *GSTT1* gene deletion is 20 percent (range: 13–26 percent) and 47 percent (range: 35–53 percent) for Caucasians and Asians, respectively.

In the liver, *GST* has been proposed to protect against hepatitis B virus-related injury, which is partly manifested

as extensive oxidative DNA damage (17). *GSTs*, in particular *GSTM1*, are also aflatoxin-metabolizing enzymes and therefore may be involved in regulating individual ability to metabolize the important aflatoxin-related hepatocarcinogen, exoepoxide. Therefore, it has been proposed that the decreased production of *GSTM1* and *GSTT1* in the null types is expected to be associated with an increased HCC risk in the presence of hepatitis B virus infection and/or aflatoxin exposure.

The most extensively studied *GSTP1* variants are exon 5 A1404G encoding an Ile→Val exchange at codon 105 (Ile105Val) (reference single nucleotide polymorphism (rs) accession identification number (rs#947894)) and exon 6 C2294T encoding an Ala→Val exchange at codon 114 (Ala114Val) (rs#1799811); both have been shown to confer lower levels of metabolic activity (18–20). The minor allele frequency of the Ile105Val variant is 31 percent, 54 percent, and 17 percent for Caucasians, African Americans, and Asians, respectively. The Ala114Val minor allele is present in about 10 percent of Caucasians but absent in African Americans and Asians (21).

Individual genetic association studies, such as those that have evaluated selected *GST* polymorphisms and risk of HCC, are frequently highly underpowered and often report small or variable effects. Meta-analysis has been recognized as an important tool to more precisely define the effect of selected polymorphisms on risk of disease and to identify potentially important sources of between-study heterogeneity (22, 23). We therefore conducted a systematic review and meta-analysis to more precisely define the effect of *GST* polymorphisms on risk for HCC.

MATERIALS AND METHODS

Eligibility criteria

We followed recognized guidelines for the conduct and reporting of meta-analyses (24). All epidemiologic studies examining the effect of *GST* polymorphisms on HCC risk within a geographically defined human population were eligible for inclusion if they had a case group with medically documented HCC, a nonrelated and comparable control group without HCC, and *GST* genotype confirmed by polymerase chain reaction or related DNA identification methods.

To minimize potential publication bias, no restrictions were placed on time period, sample size, population, language, or type of report (e.g., abstract, dissertation, or manuscript). However, given that a critical requirement in meta-analysis is the statistical independence of observations (25), when multiple reports were available for a single unique study population, we included only the most recent or largest report.

Studies were excluded if the case group consisted of unspecified liver cancer or if the control group consisted of nondiseased tissue from the cases or first-degree relatives of the HCC cases or had liver disease or cancer without adequate exclusion of possible HCC.

Search strategy

To identify all potentially eligible studies, two investigators independently conducted keyword searches in selected

databases (PubMed, OMIM, EMBASE, ISI Web of Science, and Dissertation Abstracts) and in the electronic conference proceedings for the International Genetic Epidemiology Society (1997–2005) and the American Society for Human Genetics (1999–2006). Searches included all possible combinations of selected keywords including “hepatocellular carcinoma,” “liver cancer,” or “HCC” with “genes,” “inherited factors,” “genetic polymorphisms,” “*GST*”; “glutathione S-transferase”; “*GSTA*”; “*GSTAI*”; “*GSTA2*”; “*GSTA4*”; “*GSTK*”; “*GSTKI*”; “*GSTM*”; “*GSTM1*”; “*GSTM2*”; “*GSTM3*”; “*GSTS*”; “*GSTS1*”; “*GSTT*”; “*GSTT1*”; “*GSTT2*”; “*GSTP*”; “*GSTP1*”; “*GSTO*”; “*GSTO1*”; “*GSTO2*”; “*GSTZ*”; and “*GSTZI*.” Searches were updated as of November 1, 2006.

We also used ancestry methods, including review of the bibliographies of all eligible studies and from relevant review articles, to identify additional studies not captured by our keyword searches.

Data abstraction

Two investigators independently reviewed titles, abstracts, and manuscripts identified by keyword searches to determine if an individual study was eligible for inclusion in the meta-analysis. All disagreements about eligibility were resolved during a consensus meeting with a third reviewer.

Data on study methods and findings were entered directly into a structured database. When the most recent or largest report for an eligible study population did not include sufficiently detailed information on methods, data from any earlier or smaller reports were used. When specific results were not reported, we used available tabular data to calculate them. When data were otherwise unavailable, we contacted the corresponding author by e-mail for additional information.

Analysis

The effect measure of choice was the odds ratio and associated 95 percent confidence interval. Because *GSTM1* and *GSTT1* have common deletion polymorphisms leading to absent or reduced enzymatic activity, studies have typically compared null with nonnull genotypes for HCC risk. For all other polymorphisms, planned comparisons included wild-type versus rare homozygotes and heterozygotes. We recalculated all reported odds ratios comparing differences in genotype distribution among cases and controls, as well as tests for Hardy-Weinberg equilibrium in controls, using reported tabular data. When there was a discrepancy between reported and calculated results, the tabular calculations were used for all subsidiary analyses.

We evaluated heterogeneity across studies using the I^2 of Higgins and Thompson (26), which quantifies the proportion of total between-study variation attributed to actual between-study differences or heterogeneity as opposed to random error or chance.

Our decision to perform a fixed or a random-effects meta-analysis was based on our heterogeneity assessment. When substantive between-study heterogeneity is observed, a random-effects meta-analysis (27) is the preferred method

for obtaining the pooled estimator. All meta-analyses are presented as forest plots that include odds ratios and 95 percent confidence intervals for all individual studies, as well as the pooled estimator. Shaded figures provided for all odds ratios have dimension proportional to study weight.

We decided a priori to evaluate the following variables as potential sources of heterogeneity with random-effects meta-regression: ancestry, source of the controls, sample size, date of publication, and whether the predominant nongenetic risk factor was infection with hepatitis B. We performed an analysis of influence to determine the effect of omission of individual studies on overall pooled estimators, as well as a cumulative meta-analysis by year of publication, to determine how the pooled estimator changed as evidence accumulated over time. Finally, we used Egger’s regression test, which assesses whether the relation between effect size and variance differs between large and small studies, to assess whether there was potential small-study or publication bias (28).

All analyses were conducted using STATA, version 9.0, software (StataCorp LP, College Station, Texas).

RESULTS

Searches

We identified 30 potentially eligible reports (29–58). All were case-control studies, and most were reported in English (80 percent). The majority (87 percent) were identified by structured keyword searches in PubMed. However, four reports were identified solely by ancestry methods (38, 47, 48, 54), of which three were in Chinese (38, 48, 54).

Review of abstracts and manuscripts resulted in exclusion of 15 reports because they were earlier or smaller reports from an eligible study population ($n = 11$) (44–50, 53–55, 58), included the same results presented in the eligible report ($n = 2$) (43, 52), or had an ineligible control group ($n = 2$) (56, 57).

Study characteristics

Baseline characteristics for the 15 eligible studies are provided in table 1. Fourteen evaluated *GSTM1* (29–40, 42, 51), 13 evaluated *GSTT1* (29–38, 40, 42, 51), three evaluated *GSTP1* A313G (36, 38, 51), one evaluated both *GSTO1* A140D and *GSTO2* N142D (41), and one evaluated *GSTA1*, *GSTA4*, *GSTM2*, and *GSTM3* (51).

Most of the eligible studies were performed in populations of Asian ancestry ($n = 11$). Sample size was variable though generally small, with six studies having less than 100 HCC cases and five studies with a total sample size of less than 250. More than half of all studies used hospital-based controls ($n = 8$), and many used prespecified criteria to match cases to controls ($n = 12$). A minority of studies ($n = 3$) were specifically restricted to hepatitis B carriers (31, 37, 39).

Association of GST polymorphisms and HCC

GSTM1. The 14 studies evaluating the association of the *GSTM1* polymorphism and HCC included a total of 2,514

TABLE 1. Characteristics of studies eligible for review and meta-analyses evaluating the effect of the glutathione S-transferase genotype on risk of hepatocellular carcinoma

Study, year (ref*)	Country (regions)	Sample size (cases/controls)	Study eligibility dates (month/year)	Race/ ethnicity	Case selection†	Mean age (years) of cases (SD* or %)	Male cases	
							No.	%
Kirk, 2005 (29)	Gambia (Banjul, Fajara, Bansang)	624 (216/408)	9/1997–11/2001	African	Hospital based	48.1 (15.2)	173	80.1
Covolo, 2005 (30)	Italy (Brescia, Pordenone)	600 (200/400)	3/1999–7/2002	Caucasian	Hospital based	66.5 (8.0)	158	79.0
Chen, 2005 (31)	Taiwan (Taipei, Taoyan)	966 (577/389)	9/1997–12/2001	Asian	Cohort based	52.3 (12.7)	496¶	86.0
Deng, 2005 (32)	China (Guangxi)	541 (181/360)	1/1998–12/2002	Asian	Hospital based	49 (NR*)	145	80.1
Yu, 1999 (33)#	Taiwan (Taipei)	459 (84/375)	8/1988–12/1996	Asian	Cohort based	≥60 (33.3%)	84	100
Tiemersma, 2001 (34)	Sudan (west and central)	306 (112/194)	9/1996–12/1998	African and Arab	Hospital based	57.0 (12.2)	86	76.8
Ladero, 2006 (35)	Spain (Madrid)	513 (184/329)	1/1994–12/2004	Caucasian	Hospital based	65.3 (7.0), males	150	81.5
Munaka, 2003 (36)	Japan (Kitakyushu)	216 (78/138)	6/1997–4/1998	Asian	Hospital based	66.1 (7.7)	61	78.2
McGlynn, 2003 (51)#	China (Haimen City)	487 (231/256)	2/1992–12/1993	Asian	Cohort based	55.8, males; 59.3, females	187	81.0
Sun, 2001 (37)#	Taiwan (mainland, Ponghu islets)	228 (79/149)	1991–1997	Asian	Cohort based	53 (7)	66	83.5
Liu, 2002 (38)**	China (Shanghai)	228 (84/144)	NR	Asian	Hospital based	NR	NR	
Zhu, 2005 (39)**	China (Hangzhou)	225 (91/134††)	5/2004–10/2004	Asian	Hospital based	50.2 (10.1)	79	86.8
Long, 2005 (40)	China (Guangxi)	676 (140/536)	2002–2003	Asian	Hospital based	>65 (18.6%)	111	79.3
Marahatta, 2006 (41)	Thailand (Khon Kaen)	58 (28/30)	NR	Asian	Hospital based	NR	NR	
Long, 2006 (42)¶, **	China (Guangxi)	906 (257/649)	1/2004–5/2005	Asian	Hospital based	>65 (18.3%)	208	80.9

Table continues

cases and 4,416 controls (29–40, 42, 51), with most DNA samples successfully genotyped for each polymorphism (96.7 percent). Thirteen studies solely determined the presence versus the absence of *GSTM1* (null vs. nonnull). Therefore, data were unavailable to assess Hardy-Weinberg equilibrium among controls.

The relative frequency of the *GSTM1* null genotype among control groups varied widely from 25.9 percent in ancestral Africans from The Gambia (29) to 60.2 percent in ancestral Chinese from Taiwan (37). Eight of 14 studies

showed small to modest excess risk with *GSTM1* ranging from 11 percent (35) to 147 percent (42), although the excess was statistically significant in only five studies (32, 38–40, 42).

Because substantial between-study heterogeneity was observed ($I^2 = 84$ percent), we used a random-effects model. Overall, the pooled estimator for *GSTM1* null suggested that it might convey a small excess risk for HCC, although this effect was not statistically significant (odds ratio (OR) = 1.16, 95 percent confidence interval (CI): 0.89, 1.53) (figure 1).

TABLE 1. Continued

Control selection†,‡	Type of controls	Mean age (years) of controls (SD or %)	Male controls		Matched design (criteria)	Restrictions	GST polymorphisms evaluated
			No.	%			
Hospital based	No clinical liver disease and normal α -fetoprotein levels	44.8 (15.2)	292	71.6	Frequency (gender, 10-year age group, recruitment site)		<i>GSTM1; GSTT1</i>
Hospital based	Not admitted for liver disease, cancer, or alcohol- or smoking-related disease	66.5 (8.0)	316	79.0	Frequency (gender, date,§ age \pm 5 years, recruitment site)	Caucasian, native-born Italian, aged <76 years	<i>GSTM1; GSTT1</i>
Cohort based	No liver disease per clinical, radiologic, and laboratory evaluations	53.0 (12.5)	335¶	86.0	Frequency (gender, \pm 10 years of birth)	All cases and controls HepBsAg+*	<i>GSTM1; GSTT1</i>
Hospital based	Without cancer	NR	288¶	80.0	Frequency (age, gender)		<i>GSTM1; GSTT1</i>
Cohort based	No liver disease per ultrasonography and α -fetoprotein levels	≥ 60 (30.1%)	375	100	Cases (age \pm 5 years, date,§ recruitment site)	Males only	<i>GSTM1; GSTT1</i>
Community based	Randomly selected	44.9 (10.9)	146	75.3	Frequency (gender, region)		<i>GSTM1; GSTT1</i>
Community based	Healthy per clinical and laboratory evaluations	70.4 (9.0), males	198	60.2	Unmatched (NA*)	Caucasian, Spanish ancestry and nationality	<i>GSTM1; GSTT1</i>
Hospital based	Without cancer	67.2 (10.5)	94	68.1	Unmatched (NA)		<i>GSTM1; GSTT1; GSTP1</i>
Cohort based	Healthy	NR	189	73.8	Cases (gender, age, township)		<i>GSTM1; GSTT1; GSTP1; GSTM2; GSTM3; GSTA1; GSTA4</i>
Cohort based	Without liver disease per laboratory values	53 (7)	122	81.9	Cases (age \pm 5 years, gender, date,§ residence)	All cases and controls HepBsAg+	<i>GSTM1; GSTT1</i>
Hospital based	Healthy	NR	NR		Unmatched (NA)		<i>GSTP1; GSTM1; GSTT1</i>
Hospital based	Normal liver function test without cancer, alcohol, or hepatitis B virus	48.2 (9.2)	117	87.3	Matched (age, gender)	All cases and controls HepBsAg+	<i>GSTM1</i>
Hospital based	Without personal or family history of cancer	>65 (14.7%)	384	71.6	Frequency (age, race, gender)		<i>GSTM1; GSTT1</i>
Community based	Community controls	NR	NR		Matched (race, gender)		<i>GSTO1; GSTO2</i>
Hospital based	Healthy without clinical evidence of liver disease	>65 (14.6%)	490	75.5	Frequency (age \pm 5 years, gender, ethnicity, HepBsAg)	Aged 25–75 years	<i>GSTM1; GSTT1</i>

* Ref, reference citation; SD, standard deviation; HepBsAg+, hepatitis B surface antigen positive; NR, not reported; NA, not applicable.

† Source of selection is hospital based, i.e., selected from specific hospital(s) or clinic(s); cohort based, i.e., selected from preexisting hepatocellular carcinoma or hepatitis B surface antigen cohort studies; or community based, i.e., selected from specified community(ies).

‡ Hardy-Weinberg equilibrium fulfilled in all studies.

§ Date is the date recruited, seen at recruitment location, or obtained data.

¶ Calculated from data provided in the original manuscript.

Nested case-control study.

** Foreign language.

†† Only normal control group eligible and included; two additional control groups, liver cirrhosis ($n = 58$) and chronic hepatitis B virus ($n = 63$), not included.

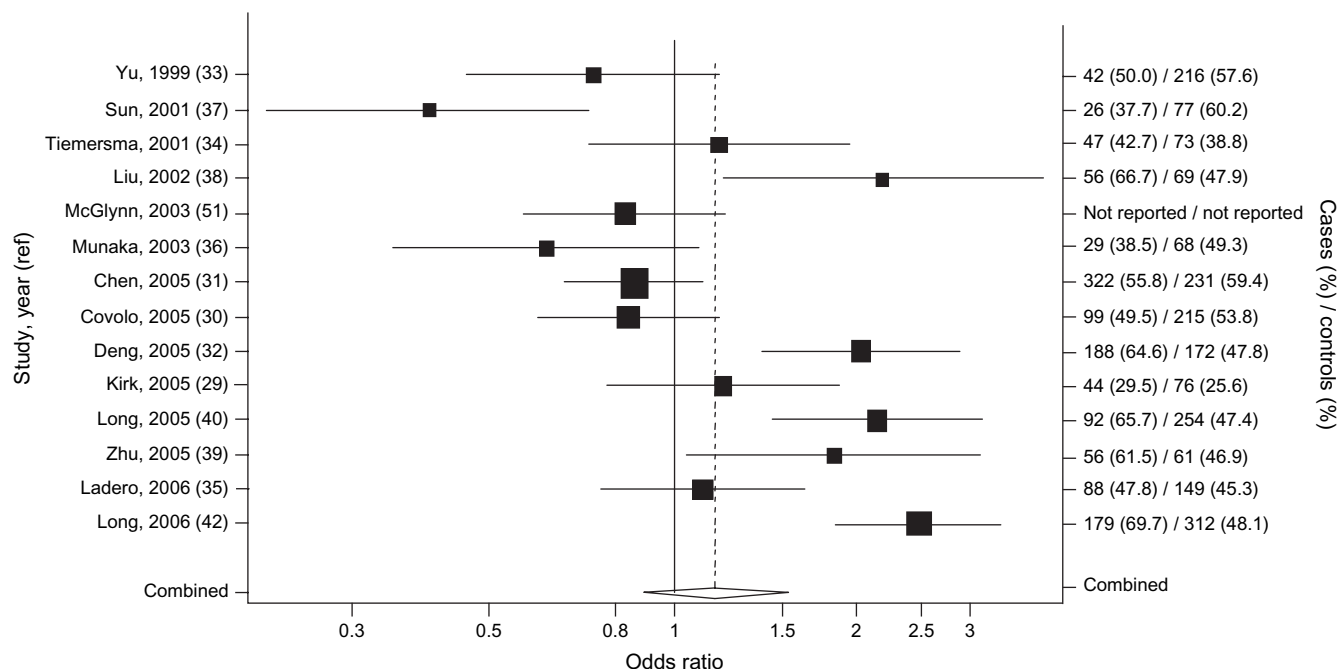


FIGURE 1. Study-specific and random-effects pooled odds ratios with 95% confidence intervals for risk of hepatocellular carcinoma with *GSTM1* null genotype. The box size is proportional to the meta-analysis study weight. Ref, reference citation.

We used random-effects meta-regression to evaluate five potential sources of between-study heterogeneity specified a priori, including the following: source of controls (hospital based vs. not), year of publication, sample size, ancestry of controls, and whether the study was restricted solely to hepatitis B carriers. Univariate analysis suggested that the source of the controls ($\tau^2 = 0.14$, $p < 0.01$) and year of publication ($\tau^2 = 0.06$, $p < 0.055$) were potentially important and explained 38 percent and 21 percent of between-study heterogeneity, respectively. However, the small number of studies prohibited further exploratory evaluation with multivariate analysis.

We found no evidence of substantive small-study or publication bias among these 14 studies ($p_{\text{Egger's}} = 0.71$). Further, our analysis of influence demonstrated that omission of any single study did not markedly change overall findings, with pooled estimators for the *GSTM1* null genotype ranging from an odds ratio of 1.25 (95 percent CI: 0.97, 1.62) to an odds ratio of 1.10 (95 percent CI: 0.85, 1.41) with the removal of the smallest (37) and largest (42) study, respectively (data not shown). Finally, our cumulative meta-analysis by year of publication showed that the pooled estimator generally trended toward a small though nonsignificant excess risk with publication of the final eight studies between 2005 and 2006 (Appendix figure 1) (29–32, 35, 39, 40, 42).

GSTT1. Thirteen studies (29–38, 40, 42, 51) with a total of 2,423 cases and 4,327 controls also evaluated *GSTT1*. As with *GSTM1*, only the presence versus the absence of the *GSTT1* polymorphism was determined, so data were unavailable to assess Hardy-Weinberg equilibrium.

The relative frequency of the *GSTT1* null genotype among control groups varied widely from 18.0 percent in Caucasians from Italy (30) to 60.2 percent in Chinese from Taiwan (31). Although a greater proportion of studies reported excess risk of HCC with *GSTT1* null ($n = 9$) (29, 31–33, 35, 36, 38, 40, 42), the excess risk was generally very small to modest, and fewer studies reached statistical significance (38, 40, 42).

As substantial between-study heterogeneity was observed with *GSTT1* ($I^2 = 63$ percent), we again used a random-effects model. Overall, the *GSTT1* null genotype conveyed a similarly small increased risk of HCC that approached significance (OR = 1.19, 95 percent CI: 0.99, 1.44) (figure 2).

Meta-regression identified the source of the controls as the only significant source of between-study variability ($\tau^2 = 0.035$, $p < 0.01$), explaining 55 percent of total observed variability. Most of the additional analyses performed for *GSTT1* had results similar to those for *GSTM1*, including the following: absence of small-study or publication bias ($p_{\text{Egger's}} = 0.49$), robustness of the pooled estimator to removal of single studies (data not shown), and a trend toward a small excess risk that approached significance with *GSTT1* null with the publication of the final seven studies between 2005 and 2006 (29–32, 35, 40, 42) (Appendix figure 2).

GSTP1. Three studies evaluated *GSTP1* A313G, and all were conducted in populations of Asian ancestry (36, 38, 51). There were a combined total of 393 cases and 538 controls, all with successfully genotyped DNA data. One study solely reported a lack of association between *GSTP1* polymorphisms and HCC ($p < 0.17$) and did not provide by-genotype data (51). Among the other two studies, one solely reported

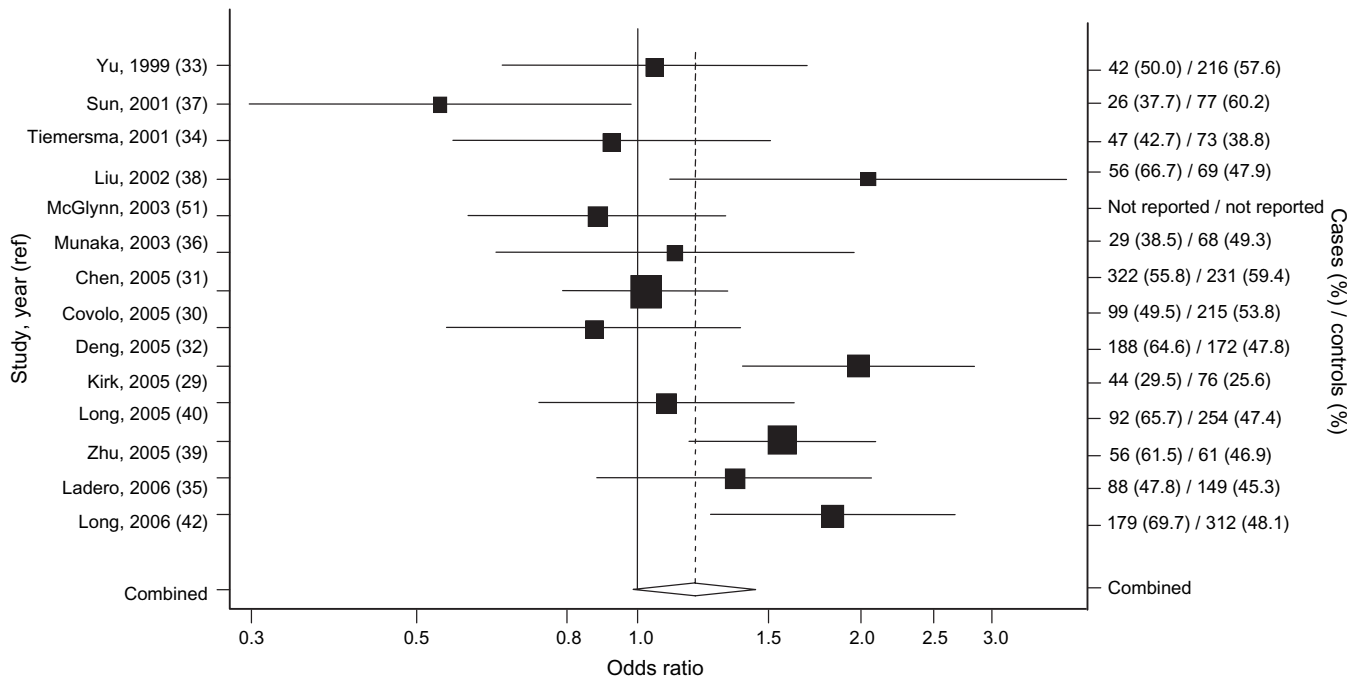


FIGURE 2. Study-specific and random-effects pooled odds ratios with 95% confidence intervals for risk of hepatocellular carcinoma with *GSTT1* null genotype. The box size is proportional to the meta-analysis study weight. Ref, reference citation.

tabular data that combined heterozygotes and rare homozygotes into a singular “high-risk” genotype (“any G”) (36). Therefore, only a similarly combined pooled estimator could be calculated, and Hardy-Weinberg equilibrium could be confirmed only in the other study. A small and nonsignificant decreased risk of HCC was observed with the high-risk genotype (OR = 0.75, 95 percent CI: 0.50, 1.15) (figure 3).

GSTO1 and GSTO2. Only one study evaluated *GSTO1* A140D and *GSTO2* N142D (41) (table 2). All 28 cases and 30 controls had DNA samples that were successfully genotyped. Both polymorphisms were reported and confirmed to be in Hardy-Weinberg equilibrium. Odds ratios were reported only for comparison of the high-risk (any “A” and any “G,” respectively) with wild genotype. Although no excess risk was observed with the high-risk genotype for *GSTO1* (OR = 0.96, 95 percent CI: 0.33, 2.80), a very large and significant excess was observed for the high-risk genotype for *GSTO2* (OR = 23.83, 95 percent CI: 5.11, 123.8).

GSTA1 and GSTA4. Only one study evaluated *GSTA1* and *GSTA4* (51). There were a total of 231 cases and 256 controls. Neither *GSTA1* nor *GSTA4* was associated with HCC in a by-polymorphism analysis ($p < 0.09$ and $p < 0.56$, respectively). A by-genotype comparison was reported only for *GSTA4*. Although there was no association between the *GSTA4* genotype and HCC risk overall (OR = 0.84, 95 percent CI: 0.58, 1.20), there was significant increased risk of HCC when the analysis was restricted to males (OR = 1.55, 95 percent CI: 1.03, 2.35).

GSTM2 and GSTM3. Finally, only one study evaluated *GSTM2* and *GSTM3* (51). There were a total of 231 cases

and 256 controls. The study population was monomorphic for *GSTM3*, and therefore no test of association was performed. There was no significant association between *GSTM2* polymorphisms and HCC ($p < 0.12$).

Interaction among GST polymorphisms. Eight studies reported assessment of interaction between *GSTM1* and *GSTT1* (32, 34–38, 40, 44). This includes seven of 13 studies that evaluated both polymorphisms in the current meta-analysis, as well as in another study (44), which (although ineligible for the meta-analysis because it was an earlier report from an eligible study population) provided unique information on the *GSTM1-GSTT1* interaction.

Excess risk of HCC with dual deletion of *GSTM1* and *GSTT1* was reported in four studies (ORs ranging from 1.46 (95 percent CI: 0.74, 2.87) (35) to 4.13 (95 percent CI: 1.70, 10.14) (38)). Further, there was evidence of significant interaction in three studies (32, 38, 40), with reported odds ratios with the dual null genotype from 1.3 to 2.0 times greater than those observed for either main effect. However, other studies reported only absence of interaction without providing specific measures of effect (34, 36, 37).

There was no evidence of interaction between *GSTP1* and *GSTM1* or *GSTT1* (36).

Interaction among GST and other genes. Eleven studies evaluated whether risk of HCC was modified by the presence of other genes (29–31, 34, 36, 42–44, 46, 49, 58). This includes several studies (43, 44, 46, 49, 58) that, although ineligible for the current meta-analysis, nonetheless provided unique data on possible interaction.

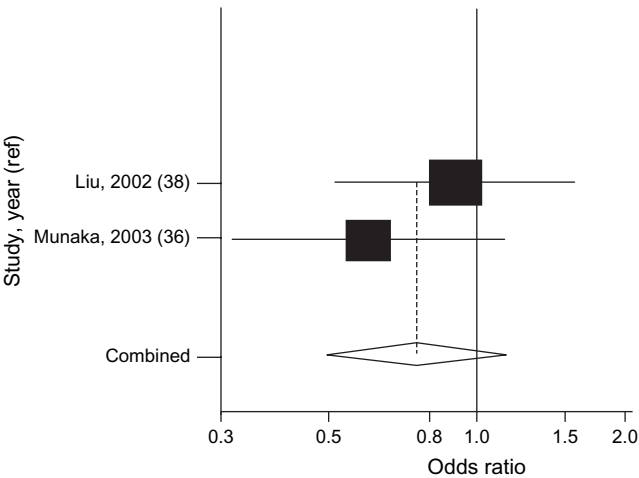


FIGURE 3. Study-specific and random-effects pooled odds ratios with 95% confidence intervals for risk of hepatocellular carcinoma with *GSTP1* A313G high-risk genotype by year of publication. The box size is proportional to the meta-analysis study weight. Ref, reference citation.

For *GSTM1*, there was no evidence of interaction with *IL-IRN* (31), *NAT2* (43), *CYP2E1* (36, 49), *L-myc* (46), *ALDH2* (36), or *ADH3* (30). Results with *HYL1**2 were equivocal, with one study reporting significant interaction between *GSTM1* null and *HYL1**2 (42) and the other finding no evidence of interaction (29). Results with *EPHX1* were contradictory: Two studies found evidence of significant interaction with *GSTM1* null (29, 58), while another found significant interaction only with *GSTM1* nonnull (34). The most suggestive finding of possible interaction was with *XRCC1*, a gene involved in DNA repair. Two of three studies found evidence of strong, significant, and dose-dependent interaction between *GSTM1* null and *XRCC1* (29, 42). However, a third study found a suggestive although much weaker and nonsignificant interaction only with *GSTM1* nonnull (44).

Fewer studies evaluated potential interactions between *GSTT1* and other genes. Evidence of significant interaction was found between *GSTT1* null and *XRCC1* (44) and between *GSTT1* nonnull and *EPHX1* (34). However, no evidence of interaction was reported for *IL-IRN* (31), *NAT2* (43), *ALDH2* (36), or *CYP2E1* (36).

There was no reported interaction between *GSTP1* and either *CYP2E1* (36) or *ALDH2* (36).

Interaction between GST and environmental exposure. Evidence suggestive of possible interaction between *GSTM1* null genotype and alcohol consumption was reported in two of three (30, 33, 36) studies, with elevated risk among heavy drinkers reported in one study (Synergism Index: 2.28) (30) and among drinkers with low plasma carotenoid levels reported in the other (33). Evidence of possible interaction between *GST* polymorphisms and smoking was also reported in two of three studies (33, 36, 43), with nonsignificant excess risk reported among light smokers with a *GSTT1* null genotype in one study (43) and a significant excess risk among smokers with

TABLE 2. Reported effects of *GSTO1* and *GSTO2* polymorphisms on risk of hepatocellular carcinoma

Study, year (ref*)	GST polymorphisms	Referent (wt*/wt)	Cases (wt/wt)		Controls (wt/wt)		Cases (heterozygous)		Controls (heterozygous)		Cases (homozygous)		Controls (homozygous)		Case/control wt gene frequency	Case/control rare gene frequency	Odds ratio	95% confidence interval
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%				
Marahatta, 2006 (41)	<i>GSTO1</i> A140D	Ala*/Ala	6	21.4	26	86.7	20	71.4	4	13.3	2	7.1	0	0	0.77/0.93	0.23/0.07	23.83	5.11, 123.8
Marahatta, 2006 (41)	<i>GSTO2</i> N142D	Asn*/Asn	18	64.3	19	63.3	7	25.0	9	30.0	3	10.7	2	6.7	0.77/0.78	0.23/0.22	0.96	0.33, 2.80

* Ref, reference citation; wt, wild type; Ala, alanine; Asn, asparagine.

a *GSTM1* or *GSTT1* null genotype and low levels of plasma beta-carotene reported in the other (33). Overall, the most frequently evaluated environmental exposure was aflatoxin B₁ (29, 34, 37, 40, 42), with evidence of significant interaction with *GSTM1* null reported in four studies (29, 34, 40, 42) and with *GSTT1* null reported in two studies (37, 40).

Laboratory testing

All studies except one (51) evaluating *GSTM1* and *GSTT1* used multiplex polymerase chain reaction to visually determine the presence versus the absence of the polymorphism. Studies evaluating *GSTP1*, *GSTO1*, *GSTO2*, *GSTM3*, *GSTA1*, and *GSTA2* used polymerase chain reaction/restriction fragment length polymorphism to identify specific polymorphic variants.

Although most reports contained detailed information on the methods used for DNA isolation and on the use of specific primers and internal controls, few reported explicitly on information related to other aspects of quality control including the following: performance of duplicate testing (two studies only (34, 37)), use of a single laboratory to perform DNA testing (two studies only (29, 37)), blinding of laboratory personnel to disease status (two studies only (33, 37)), and timing of testing, including whether matched case and control DNA samples were processed at the same time (one study only (37)).

DISCUSSION

This report is the first published meta-analysis specifically examining the effect of *GST* polymorphisms on risk of HCC. We found that null variants of *GSTT1* and, to a lesser extent, *GSTM1* might be associated with a small increase in HCC risk. Although considerably less examined, there was no evidence for an association between *GSTP1* and HCC with insufficient evidence to make a statement for the other *GST* variants evaluated. There were also a number of potentially interesting gene-gene and gene-environment interactions reported in individual studies. However, these were too few and too inconsistent to allow a meta-analysis. All of the studies identified for possible inclusion in this meta-analysis were conducted in populations with high (Asian, African) or medium (European) incidence rates for HCC, with most conducted in Asian populations (11 of 15 studies) and in countries where hepatitis B is endemic (12 of 15 studies).

There is currently a remarkable absence of studies on *GST* and HCC from low-incidence regions including North America, where HCC incidence has more than doubled over the past two decades (59), and neither hepatitis B nor aflatoxin exposure is a major etiologic risk factor for HCC. Oxidative stress is proposed to be an important pathogenic factor in liver damage related to alcohol and hepatitis C, the major risk factors for HCC in these regions, either alone or in combination with other factors including obesity-related nonalcoholic fatty liver disease. Further, there is preliminary evidence indicating that *GST* polymorphisms are associated with increased risk of advanced liver disease that serves as a precursor for HCC in the setting of alcoholic liver disease. One study from the United Kingdom compared the fre-

quency of *GST* polymorphisms in patients with alcoholic liver disease from heavy drinking and normal local controls. A significantly increased prevalence of the *GSTT1* null genotype was observed in alcoholic liver disease patients in comparison with nondrinking controls (OR = 2.1, 95 percent CI: 1.1, 4.7) (60). Another study from Finland reported that homozygous deletion of the *GSTM1* gene may indicate increased susceptibility to irreversible liver damage in response to the toxic effects of ethanol. In that study, the *GSTM1* null genotype indicating absent enzymatic activity was assessed in 33 abstainers, 43 moderate alcohol consumers, and 313 heavy alcohol consumers; the study found that the null genotype was nearly significantly more frequent among heavy consumers with at least slight liver fibrosis ($p = 0.05$; OR = 1.8) and statistically significant more frequently among alcoholics with advanced liver fibrosis ($p < 0.025$; OR = 2.3) (61). Finally, nonalcoholic fatty liver disease has become the most common liver disorder in several countries in North America and Europe (62). A study from Italy analyzed the catalytic activities of *GST* in the blood of 21 children with nonalcoholic steatohepatitis and 28 controls. *GST*, which provides a second defense line against oxidative stress, was 17.8 percent increased in cases compared with controls (63). Similar arguments can be made for hepatitis C, where GSTs are involved in the metabolism of endogenously generated, cancer-causing reactive oxygen species continuously produced through hepatitis C-induced inflammatory disease. In summary, the evidence of a potential role of *GST* polymorphisms in liver disease related to common risk factors in low-risk populations in North America and Europe justifies further examination of these *GST* polymorphisms as risk factors for HCC in these currently unstudied and understudied regions.

Among the medium- and high-incidence populations where studies evaluating *GST* polymorphisms have been performed, we identified a total of 14 eligible studies evaluating *GSTM1* (13 of which also evaluated *GSTT1*) (29–40, 42, 51). *GSTM1* and *GSTT1* protect cells from the natural by-products of lipid peroxidation and oxidative stress; deletion of the *GSTM1* and *GSTT1* genes is associated with enhanced endogenous mutagenic processes that are implicated in susceptibility to other inflammation-related cancers of the gastrointestinal tract, such as pancreatic cancer (64, 65). The overall results of our random-effects meta-analyses suggest a small excess risk of HCC with *GSTT1* (OR = 1.19, 95 percent CI: 0.99, 1.44) and possibly *GSTM1* (OR = 1.16, 95 percent CI: 0.89, 1.53) null genotypes. The pooled estimators generally trended toward a small excess risk with publication of the most recent studies.

We also performed meta-regression to evaluate the effect of several preselected factors on the observed variability among studies evaluating *GSTM1* or *GSTT1* null genotype. Univariate analysis suggested that the source of controls and possibly the year of publication and hepatitis B predominance may be important sources of between-study heterogeneity. However, these analyses are limited by multiple comparisons, the assumption that the association found at the study level applied at the individual level (66), and our inability to perform multivariate analyses given the small number of studies.

GSTP1 was evaluated in only three studies (36, 38, 51). All reported no association with HCC, although only two provided by-genotype data that could be combined in a pooled estimator (OR = 0.75, 95 percent CI: 0.50, 1.15). These results suggest that *GSTP1*, combined with the fact that its greatest expression is in the brain and not the liver, is unlikely to be a strong independent risk factor for HCC.

Only one study evaluated each of *GSTO1* (41), *GSTO2* (41), *GSTA1* (51), *GSTA4* (51), *GSTM2* (51), and *GSTM3* (51). Significantly increased risk of HCC was observed with *GSTO2* (41) and with *GSTA4* in men only (51). However, these results should be considered as provisional, because results of the earliest genetic association study tend disproportionately to report the strongest or most significant findings and are frequently not replicated by subsequent research (67, 68).

The current meta-analysis has several strengths, most notably a rigorous design that included structured searches in multiple databases for published reports, unpublished abstracts, and dissertations along with the use of ancestry or bibliography searches to identify nondatabase-indexed reports. These efforts, as well as our inclusion of non-English language reports, were important in minimizing a major potential threat to the validity of any meta-analysis—publication bias and the related threat of a language bias. Another important strength is our performance of auxiliary analyses, including meta-regression and cumulative meta-analyses, that allowed a more thorough examination and appropriate qualification of our results.

Our meta-analysis is, however, subject to the same potential limitations that affect all meta-analyses, including the direct comparability and quantity and quality of available reports. The issue of comparability is of lesser concern here, given the nature of our exposure (inherited genotype) and outcome (medically confirmed HCC) and the uniform use of a case-control design.

Limited sample size is of concern in the current meta-analysis. Only 14 eligible studies evaluated *GSTM1* null, the most frequently studied *GST* variant, with a total sample size of 966 in the largest study and with five studies having a total sample size of less than 250. It is well accepted that the strength of an association is not an inherent biologic property with small associations potentially reflecting important causal relations (69). However, the following three points have only recently become understood: 1) It may take relatively few common genetic variants, each conveying only small to modest excess risk, to account for a sizable portion of the population attributable fraction for common diseases (e.g., 10–18 genes, each with a 20–30 percent prevalence and conveying an odds ratio of only 1.2–1.5 to explain between 30 and 50 percent of the population attributable fraction) (70); 2) there is meta-analytical evidence suggesting that some common genetic variants do convey small to modest excess risk for several common diseases (e.g., *DRD3* and schizophrenia and *PPARG* and type II diabetes) (71); and 3) the minimum sample size needed to reliably detect the likely small individual effects of common genetic variants contributing to common chronic diseases is much larger than previously thought (i.e., often multiple thousands) (72). Given our limited total sample size and small observed effects, performance of ad-

ditional larger genetic association studies with subsequent update of the current meta-analysis will be needed to confirm our preliminary suggestive findings for the common deletion polymorphisms for *GSTT1* and *GSTM1*.

It is difficult to assess how variable individual study quality may have influenced the overall findings of our review. Although the reporting was generally good among individual studies, for more technical aspects of study design and conduct, the reporting was generally more limited and variable regarding the use of quality control measures such as blinding of laboratory personnel to disease status or evaluations of possible biases, particularly regarding the selection and participation of controls. Although some meta-analysts have provided quality scores (73), these scores have also been criticized because of their potential to introduce bias of unknown dimensions (74). Because we could not determine reliable or valid methods to distinguish between “absence of explicit reporting” and “absence of actual performance” of these quality control procedures, we elected not to assign quality scores and instead to provide the reader with collective data to evaluate.

In conclusion, taken together, our findings suggest a possible small excess risk of HCC with the *GSTT1* null genotype and possibly also with the *GSTM1* null genotype in populations with high or medium incidence of HCC. Larger studies will be needed to explore potential interactions between *GST* polymorphisms and other genetic and environmental risk factors for HCC in these high- and medium-risk populations. We suggest that future studies might also be conducted in previously unevaluated populations, including low-risk populations from North America and Europe, in which HCC incidence has more than doubled over the last two decades (59) and where important risk factors, such as alcohol drinking and obesity-related nonalcoholic fatty liver disease, acting either alone or in combination with hepatitis C, are major sources of oxidative stress.

INTERNET SITES

- Human Genome Epidemiology Network prepared by the National Office of Public Health Genomics, Centers for Disease Control and Prevention (<http://www.cdc.gov/genomics/hugenet/default.htm>)
- Online Mendelian Inheritance in Man by The Johns Hopkins University (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>)
- Information Hyperlinked over Proteins (<http://www.ihop-net.org/UniPub/iHOP/>) (75)
- American Society of Human Genetics (<http://www.ashg.org/genetics/ashg/menu-about.shtml>)
- International Genetic Epidemiology Society (<http://iges.biostat.wustl.edu/>)

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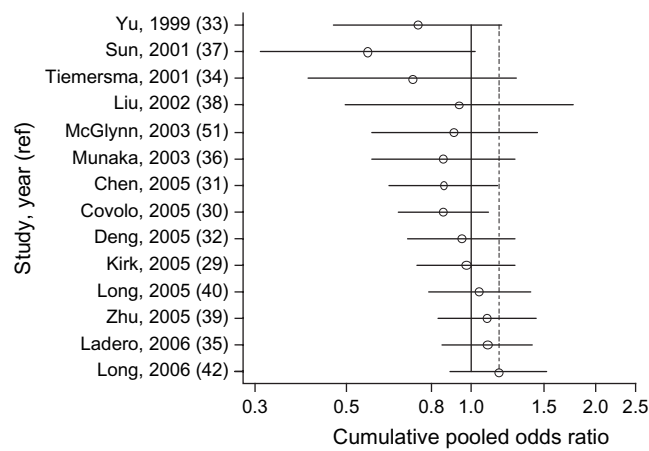
Conflict of interest: none declared.

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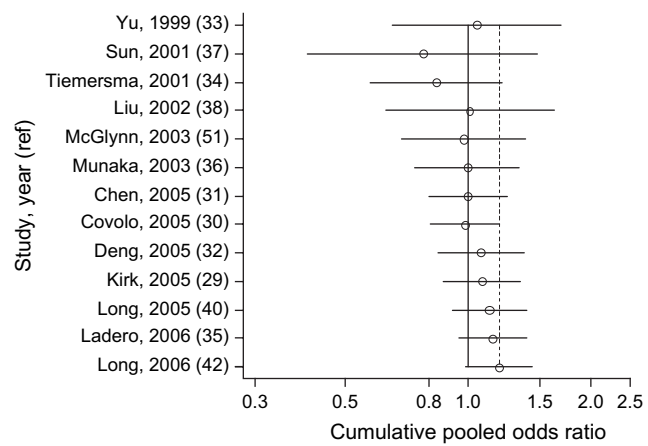
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APPENDIX



APPENDIX FIGURE 1. Cumulative meta-analysis of risk of hepatocellular carcinoma with *GSTM1* null genotype by year of publication. Ref, reference citation.



APPENDIX FIGURE 2. Cumulative meta-analysis of risk of hepatocellular carcinoma with *GSTT1* null genotype by year of study publication. Ref, reference citation.